Title

Prognostic value of serum HE4 levels and Risk of Ovarian Malignancy Algorithm (ROMA) scores at the time of ovarian cancer diagnosis.

Abstract

Objectives: To assess whether pretreatment serum HE4 levels or ROMA scores at time of initial diagnosis are associated with progression-free (PFS) and disease specific survival (DSS) in patients with ovarian cancer receiving either primary debulking surgery (PDS) or neo-adjuvant chemotherapy (NACT) followed by interval debulking surgery.

Methods: Survival analysis of 101 cases of invasive ovarian cancer recruited in a previous diagnostic accuracy study conducted from 2005-2009 at the University Hospitals KU Leuven, Belgium. Serum HE4 (pM) and Risk of Ovarian Malignancy Algorithm (ROMA) scores (%) were obtained prior to primary treatment. Dates of death were obtained by record linkage with patient hospital files. Progression was evaluated according to RECIST criteria. Adjusted hazard ratios (HR) were estimated using multivariable Cox regression. **Results:** 80 patients (79%) with invasive ovarian cancer underwent PDS whereas 21 (21%) received NACT. Median DSS was 3.72 years; 95% CI: 3.19-4.07. 52 (51%) patients died of disease, and 74 (73%) patients had progressive disease during follow-up. On univariable analysis, pretreatment HE4 levels and ROMA scores were related to worse prognosis. However, after adjustment for classical prognostic variables, HE4 levels (log2-transformed) and ROMA scores were unrelated to DSS (HE4: adjusted HR 1.01, 95% CI 0.84-1.21; ROMA: adjusted HR per 10% increase 0.96, 95% CI 0.84-1.12) and PFS (HE4: adjusted HR 0.98, 95% CI 0.84-1.13; ROMA: adjusted HR per 10% increase 0.98, 95% CI 0.88-1.11).

Conclusions: Pretreatment HE4 levels and ROMA scores are not independent prognostic factors for DSS and PFS after multivariable adjustment in patients with ovarian cancer.

Key words: ovarian cancer, biomarkers, prognosis, mortality, survival analysis.

Introduction

Ovarian cancer is a leading cause of gynecologic cancer-related death, with an age-adjusted mortality rate of 8.1 per 100.000 women per year. It is estimated that 22,240 women would be diagnosed with ovarian cancer in 2013 in the United States. Most of them will be confronted with advanced stage disease and eventually more than two thirds of patients will die from its disease course.[1]

Currently physicians widely use the staging system of the International Federation of Gynecology and Obstetrics (FIGO) to counsel individual patients regarding their prognosis.[2] Moreover, ovarian cancer is now being regarded as a heterogeneous disease entity with different appearances, biological and genetic backgrounds and concomitant clinical features.[3] This causes difficulties in predicting progression-free (PFS) and overall survival (OS) for individual patients. Other factors besides FIGO staging have a clear prognostic significance in patients with epithelial ovarian cancer. Many of them have already been incorporated into different clinical nomograms.[4-6] These include age; performance status; American Society of Anesthesiologist (ASA) score; tumor grade; histology; FIGO stage; residual tumor volume after cytoreductive surgery; serum alkaline phosphatase; serum albumin; hemoglobin level; platelet count; the presence or absence of ascites; BRCA status; and various molecular markers.[4-6] In recent years there has been an increasing interest in the prognostic value of human epididymis-protein 4 (HE4), a new molecular biomarker that is overexpressed mainly in serous and endometrioid epithelial ovarian cancer.[7] This epithelial protein has already been shown to be an independent prognostic factor for reduced OS and progressive disease in other malignancies such as endometrial, lung and breast cancer.[8-10]

However, the prognostic value of serum HE4 for these clinical outcomes in patients with ovarian cancer has yet to be fully explored. Inconsistent data exist in the literature based only on studies limited to patients with epithelial ovarian cancer scheduled for primary debulking surgery (PDS) with adjuvant chemotherapy.[11-18]

Following the identification of serum HE4, most clinical research has focused on its usefulness for the diagnosis of ovarian cancer and more specifically for risk stratification in combination with serum CA 125 as the Risk of Ovarian Malignancy Algorithm (ROMA) in women presenting with a pelvic mass.[19] Although this risk index has been optimized for use as a diagnostic tool, high ROMA scores have also been reported to be independently associated with an unfavorable prognosis in a selected group of patients with ovarian cancer.[14]

In this study we aimed to further investigate the independent prognostic importance of pretreatment serum HE4 levels and ROMA scores at time of the initial diagnosis of ovarian cancer with respect to PFS and disease-specific survival (DSS) in a population of patients with ovarian cancer receiving either PDS or neo-adjuvant chemotherapy (NACT) with interval debulking surgery.

Materials and Methods

Study cohort: In this retrospective time-to-event analysis we included all consecutive newly diagnosed cases (n=101), of biopsy proven, FIGO stage I-IV invasive ovarian cancer that received either PDS or NACT from a previous diagnostic accuracy study (ProDoC) conducted at the University Hospital KU Leuven, Belgium. This study had been approved by the Ethics Committee of the University Hospital KU Leuven (reference: OG032/ML3132). The methodology for this study has been described in detail elsewhere.[20] All included patients gave written informed consent before plasma and tissue samples were collected. For this study, data (n=389) was collected prospectively from August 2005 to March 2009. It enrolled

a total of 161 malignant (31 borderline ovarian tumors, 102 invasive ovarian cancers, 2 uterine sarcomas and 26 metastatic tumors to the ovary) and 228 benign tumors. We excluded one case of invasive ovarian cancer from our survival analysis as this patient did not receive any form of medical treatment.

The final study cohort (n=101) comprised 80 patients (79%) who underwent PDS by gynecologic oncologists with maximal effort to resect all tumor tissue present. The majority of these patients (92%) received an adjuvant first-line platinum-based chemotherapy regimen after PDS. In total five patients with stage I, well-differentiated invasive cancers did not receive adjuvant treatment.

Neo-adjuvant platinum-based chemotherapy was initiated in 21 patients (21%) all with bulky FIGO stage IIIC-IV disease. Histology (tumor type, grade) was derived before initiation of NACT either by imaging-guided biopsies (n=1), during prior diagnostic laparoscopy to assess primary resectability of the disease (n=19) or explorative laparotomy (n=1). Interval debulking surgery was performed in only 14 out of 21 patients (67%) after hematological recovery, but within 6 weeks of completion of the third chemotherapy cycle. The first cycle of chemotherapy after surgery was administered as soon as possible, but no more than six weeks later.

Assessment of progression-free and disease-specific survival: Follow-up visits (physical examination and serum CA125) were scheduled every three months during the first two years following completion of chemotherapy, then semiannually for three years and yearly thereafter. Imaging (i.e. computed tomography (CT) abdomen (and if applicable CT thorax)) was used whenever there was a clinical suspicion of disease progression and assessed according to the Response Evaluation Criteria in Solid Tumours (RECIST) criteria.[21,22]

Progression free survival was measured from start of primary therapy (i.e. date of PDS or first NACT) until the documented date of first relapse, death from any cause, or the date of last

contact (until 31 December 2012) in hospital record files for patients that were still alive and progression-free.

Disease specific survival was defined as the time interval between the start of treatment and the date of death related to ovarian cancer as documented in the hospital record files. Competing risk events (i.e. death from other causes) not directly related to ovarian cancer were censored. The last date of documented follow-up up until 31 December 2012 was used to censor surviving patients (both progression-free and relapsed patients).

Most treated patients (either progression-free or relapsed) attained careful follow-up in our center. For some patients completeness of survival data was ensured by clinical trial nurses who contacted general practitioners or treating oncologists in other hospitals every three to four months.

Serum samples, biomarker assays, and ROMA: Blood samples were obtained prior to first-line standard therapy (PDS or NACT) and collected in 10 ml clotting activating tubes (BD Vacutainer Serum Tube, ref. 369033; Becton-Dickinson, Erembodegem, Belgium). Serum tubes were centrifuged at 800 g for 10 min. Serum was collected, dispensed into multiple cryotubes and frozen at −80 °C. Serum CA125 concentrations were measured using the CanAg CA 125 EIA assay (Fujirebio Diagnostics, Göteborg, Sweden) and serum HE4 concentrations were measured using the HE4 EIA assay (Fujirebio Diagnostics). Both assays are solid-phase, non-competitive immunoassays, based on the direct sandwich technique, and were run according to manufacturer's instructions. Each ELISA was performed manually and in duplicate for calibrators, controls and patient samples. The appropriate controls were within the ranges provided by the manufacturer for all runs.

The ROMA algorithm classifies patients as being at a low or at a high risk for malignant disease using the following algorithms and thresholds according to the manufacturer’s insert:

for premenopausal women the linear predictor z equals −12.0 + (2.38 × LN(HE4)) + (0.0626 × LN(CA125)); for postmenopausal women z equals −8.09 + (1.04 × LN(HE4)) + [0.732 × LN(CA125)). The probability of malignancy equals 1/ (1 + exp (-z)).

Statistical analysis: Statistical analysis was conducted according to the reporting recommendations for tumor marker prognostic studies (REMARK)[23] and performed with R version 2.15 (www.r-project.org) and Medcalc, version 12.3.0.0, (MedCalc Software, Mariakerke, Belgium). To analyse the association of serum HE4 and ROMA scores with other prognostic variables we used the difference between medians with its 95% confidence interval (CI) (Bonett & Price, Psychological Methods 2002). For descriptive purposes, median values of serum HE4 and ROMA were used to indicate low– and high-risk groups. For descriptive purposes, overall and risk-group-stratified survival rates were estimated with the Kaplan–Meier method.

Time-to-event analysis was performed using classical Cox proportional hazards regression. First, univariable models were examined using either HE4 or ROMA scores as predictor of survival. Then, we fitted multivariable models containing classical prognostic variables, treatment type, as well as either serum HE4 or ROMA scores. In all Cox models, HE4 and the ROMA score were included as continuous variables and thus were not dichotomized. As co-variables we considered age (continuous), FIGO stage (stage III-IV versus stage I-II), tumor grade (2-3-high grade not-other-specified (NOS) versus 1), histology (serous versus non-serous), residual tumor load after surgery[24] (residual disease versus no macroscopic disease), presence of ascites (yes versus no), World Health Organization (WHO) performance status (categorical, I to III) and treatment type (NACT versus PDS). Main interest was in the crude (univariable) and adjusted (multivariable) hazard ratios (HR) for serum HE4 and the ROMA score. The proportional hazards assumption for the predictors was verified using Scaled Schoenfeld residuals. Only serum HE4 did not fulfill the linearity assumption, which was

checked by means of the Martingale residuals. After log-transformation, this assumption was fulfilled.

To account for the small number of events (i.e. death, progression) we also computed penalized hazard ratios (HR) that were based on a multivariable Cox model using a ridge penalty. This method avoids overoptimistic effects of the predictor variables. The penalization parameter lambda was derived using 10-fold cross-validation. This analysis was done in R (www.r-project.org) using the glmnet package.[25]

Results

The clinico-pathological features of the 101 patients with invasive ovarian cancer are summarized in **Table 1**. Most patients (74%) presented with stage III-IV disease and had serous ovarian cancer (67%). A complete debulking to no macroscopic residual tumor was achieved in 86% of patients that received PDS or interval debulking surgery. In patients treated with PDS (73/80) 91% were debulked to no residual disease. In patients that were debulked after NACT (8/14) 57% were debulked to no residual disease. Those patients that could not receive interval debulking surgery (7/21) after 3 cycles of NACT were all classified as having residual tumor burden. Patients receiving NACT and interval debulking surgery were more likely to be older, have a lower WHO performance status, higher FIGO stage, and serous type cancer. Median levels (interquartile range) of pretreatment serum HE4 and ROMA were 274 (pM) (96-699), and 90% (58-98), respectively.

We observed higher pretreatment HE4 levels and higher ROMA scores, in patients with advanced FIGO stage, with grade 2-3 or high grade NOS tumors, serous histology, ascites and residual tumor load after primary treatment (**Table 2**).

The median overall survival time was 3.7 years (95% CI: 3.2-4.1) for the entire study cohort. Fifty-two patients (52%) died of their disease during follow-up. One additional patient died of

a bowel perforation with peritonitis unrelated to ovarian cancer and this case was censored. Median follow-up time until first progression was 1.7 years 95%CI (1.3-1.9). Seventy-four (74%) cases had progressive disease after primary therapy.

Univariable analysis suggested that higher serum HE4 levels and ROMA scores were associated with worse prognosis for PFS and DSS (**Figure 1**). **Table 3 and 4** represent the full multivariable Cox model for PFS and DSS with adjusted HRs for each predictor variable. After controlling for known prognostic variables and treatment type no independent associations of pretreatment serum HE4 (adjusted HR for each doubling in HE4 level = 0.98, 95% CI 0.84 to 1.13) or of ROMA scores (adjusted HR for each 10% increase in risk = 0.98, 95% CI 0.88 to 1.11) with PFS were suggested. Similar results were obtained for DSS; the adjusted HR for each doubling in the HE4 level was 1.01 (0.84 to 1.21), the adjusted HR for each 10% increase in ROMA score was 0.96 (0.84 to 1.12). Penalized Cox regression reduced the effect size (HR) to PFS and DSS for most predictor variables.

Discussion

This study investigated the prognostic value of serum HE4 and ROMA scores at the time of initial diagnosis in patients with invasive ovarian cancer treated with either PDS or NACT. On univariable analysis our data suggested that patients with elevated levels of serum HE4 or ROMA scores had an unfavorable PFS or DSS. However, this association is very likely to be explained by other prognostic variables since the effect sizes disappeared after multivariable adjustment for classical prognostic variables.

The strengths of this study include the use of prospectively collected data from a previous diagnostic accuracy study; adequate steps taken to ensure completeness of survival data; and the inclusion of patients with ovarian cancer that received either PDS or NACT to support the generalizability of our findings and minimize selection bias. Moreover, NACT followed by

interval debulking is clinically used as a valuable alternative for patients with advanced stage, bulky disease as it is not inferior to PDS followed by adjuvant chemotherapy.[26] Previous studies reporting on the prognostic value of serum HE4 in patients with epithelial ovarian cancer were limited to those treated with PDS.[11-18] Patients that received NACT followed by interval debulking were all excluded from further analysis as it was suggested that this type of treatment might affect the assessment of FIGO staging, tumor type and tumor grading.[13] Since most patients that received NACT in our study underwent a prior diagnostic laparoscopy to evaluate disease extent and to obtain histology, we believe we have minimized the risk of introducing bias.

There are some limitations to address to this study. The small number of events in this study limits the number of predictor variables that can be entered in the Cox model. In addition thel use of penalized Cox regression aims to avoid overoptimistic effect sizes (hazard ratios). As shown this led to a reduction in the effect size for most variables. Another possible limitation to this study could be that we decided to report the prognostic significance of serum HE4 in relation to PFS and DSS, whereas other studies also explored the predictive value of a prognostic risk index composed of serum HE4 and CA 125 using receiver-operating characteristics (ROC) curve analysis for secondary outcomes like platinum resistance and residual tumour burden.[11, 12] This risk index combining CA125 (cut off value 500 IU/ml) and HE4 (cut off value 500 pM) was an independent predictive factor for surgical outcome in a multivariable setting (OR = 6.041, 95% CI 2.33 to 15.65).[11] Another study has showed an improved positive predictive value (PPV) in relation to platinum resistance for the combination of markers (cut-off upper third centile for both CA 125 and HE4) than for each marker individually.[12]

Instead, we evaluated the combination of both markers in the context of the diagnostic ROMA algorithm. It is important to emphasize that this risk score has been developed and

optimized only to differentiate between the benign or malignant nature of an adnexal mass and not for predicting disease outcome where its utility has only been explored in one study.[14] In contrast to our findings, in this study elevated ROMA scores were independently associated with impaired PFS (2.76; 1.15-6.59) and OS (3.22; 1.21-8.57).[14] However, this recruitment was limited to patients with epithelial ovarian cancer treated with PDS, the study had a short median follow-up time (19 months), excluded almost 10% of patients from survival analysis due to incomplete follow-up, and did not use RECIST criteria to define progressive disease.[14]

Our findings with respect to the prognostic relevance of pretreatment serum HE4 were in line with a large study conducted by the OVCAD consortium.[11] This study with a median follow-up time of 25 months (range 1–49 months), in which 83 patients (30%) died during follow-up and 182 patients developed a recurrence, also found that serum HE4 was not an independent prognostic factor for PFS and OS. However, in contrast to our design, they excluded early stage disease due to its excellent prognosis and used serum HE4 as a categorical variable in their multivariable survival analysis.[11]

Most initial reported results of prognostic studies dealing with serum HE4 showed great promise.[12-18] High levels of serum HE4 proved to be strong and independent indicators of a worse prognosis in patients with epithelial ovarian cancer, or only in those with advanced disease.[17] Our results contradict these reports. Whilst on univariable analysis both HE4 and ROMA were related to unfavorable prognosis, multivariable analysis shows they were not independent predictors.

A variety of problems are likely to explain discrepancies in outcomes of prognostic research, such as general methodological differences, poor study design, assays that are not standardized or lack reproducibility, and inappropriate or misleading statistical analyses.[27]

There are clear differences between studies exploring the prognostic value of serum HE4 including sample size, the median length of follow-up, the total number of events, and the type of prognostic variables entered in multivariable survival analysis. In addition, most studies selected patients with epithelial ovarian cancer[11-18], one study focused only on serous type cancer[12], and many did not use CA 125 or RECIST criteria[21, 22] to define progressive disease or were unclear about how to define this clinical outcome.[14,15,17] Furthermore, two studies did not use serum HE4 levels at time of diagnosis, but in contrast analyzed samples taken after PDS.[12,16] To overcome this considerable heterogeneity, it is essential that methodology for prognostic studies on serum HE4 should be more harmonized as this will only facilitate future data-synthesis in systematic reviews.

To conclude, whereas biomarkers such as serum HE4 or biomarker algorithms like ROMA are attractive tools in ovarian cancer diagnostics, this study provides evidence of their limited ability to indicate risk groups for worse overall prognosis in patients with ovarian cancer treated either by primary surgery or neo-adjuvant chemotherapy, when taking classical clinico-pathological factors into account.

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Table 1. Clinico-pathological characteristics in patients (n=101) with invasive ovarian cancer.

Characteristic	Result
Age (years), median (IQR)	60 (52 – 67)
FIGO stage	
I	20 (19.8%)
II	6 (5.9%)
III	61 (60.4%)
IV	14 (13.9%)
Malignant Histology	
Serous	68 (67.3%)
Mucinous	7 (6.9%)
Clear cell	6 (5.9%)
Carcinosarcoma	4 (4.0%)
Endometroid	7 (6.9%)
Mixed	5 (5.0%)
Undifferentiated	2 (2.0%)
Sex cord stromal tumor	2 (2.0%)
Tumor grade differentiation	
1	13 (12.9%)
2	14 (13.9%)
3	71(70.3%)
High grade NOS	3 (2.9%)
Ascites	
Yes	52 (51.5%)
No	49 (48.5%)
WHO	
0	42 (41.6%)
1	54 (53.5%)
2	5 (4.9%)
Residual tumor load	
Yes	21 (20.8%)
No	80 (79.2%)
Preoperative biomarker and ROMA levels	
Serum CA125 (IU/mL), median (IQR)	541 (104-1381)
HE4 (pM), median (IQR)	274 (96-699)
ROMA score (%),median (IQR)	90 (58-98)

Abbreviations: NOS: not other specified; IR: interquartile range; ROMA: risk of ovarian malignancy algorithm, FIGO: International Federation of Obstetrics and Gynecology.

Table 2. Median levels of serum HE4 and ROMA scores according to standard prognostic factors in women with invasive ovarian cancer.

		HE4 (pM)		ROMA (%)	
Prognostic factor	**N**	**Median**	**Difference (95% CI)**	**Median**	**Difference (95% CI)**
FIGO stage					
I-II	26	87.8	339 (169; 509)	48.2	47 (22; 71)
III-IV	75	426.8		94.9	
Histology					
Non-serous	33	102.1	342 (160; 524)	60.0	35 (12; 95)
Serous	68	443.9		95.5	
Tumor grade					
(1)	13	119.9	163 (-71; 397)	74.5	17 (-17; 50)
(2-3-high grade NOS)	88	282.9		91.1	
Residual tumor load after primary treatment					
No	81	217.5	359 (154; 565)	86.2	11 (1; 22)
Yes	20	576.9		97.4	
Presence of ascites					
No	49	138.2	365 (163; 567)	67.8	27 (8; 46)
Yes	52	503.6		94.9	

Abbreviations: ROMA: risk of ovarian malignancy algorithm, FIGO: International Federation of Obstetrics and Gynecology, NOS: not other specified.

Table 3. **Expanded Cox Model for Progression Free Survival (PFS) and Disease-specific survival (DSS) including classical prognostic variables, treatment type and serum HE4.**

	PFS			DSS		
Prognostic variable	HR	95% CI	Penalized HR	HR	95% CI	Penalized HR
HE4, per doubling	0.98	0.84-1.13	1.02	1.01	0.84-1.21	1.02
Age, per 10 years	0.92	0.72-1.17	0.98	1.16	0.86-1.58	1.13
WHO score 1 vs 0	1.13	0.66-1.96	1.08	1.01	0.51-2.03	1.00
WHO score 2 vs 0	4.11	1.22-11.9	2.95	7.43	2.02-23.8	4.97
Serous vs non-serous	1.19	0.59-2.55	1.27	0.63	0.28-1.50	0.79
Differentiation grade 2/3/high grade NOS vs 1	5.42	1.91-22.8	2.67	5.74	1.62-36.7	2.60
FIGO stage III/IV vs I/II	3.50	1.34-10.5	2.09	2.06	0.66-7.99	1.74
Residual tumor load (yes vs no)	3.09	1.41-6.87	2.29	5.76	2.52-13.6	3.71
Treatment (NACT vs PDS)	1.58	0.68-3.53	1.51	1.40	0.58-3.28	1.46
Presence of ascites	1.60	0.96-2.76	1.49	2.73	1.41-5.60	1.92

HE4 was log2-transformed such that the effect can be expressed per doubling of the marker level.

Penalized results were based on a multivariable Cox model using a ridge penalty, where the penalization parameter lambda was derived using 10-fold cross-validation.

Abbreviations: FIGO: International Federation of Obstetrics and Gynecology, WHO: world health organization, NOS: not other specified, NACT: neo-adjuvant chemotherapy, PDS: primary debulking surgery, DSS: disease specific survival, PFS: progression free survival, HR: hazard ratio .

Table 4. **Expanded Cox Model for Progression Free Survival (PFS) and Disease-specific survival (DSS) including prognostic variables, treatment type and ROMA.**

	PFS			DSS		
Prognostic variable	HR	95% CI	Penalized HR	HR	95% CI	Penalized HR
ROMA risk, per 10%	0.98	0.88-1.11	1.01	0.96	0.84-1.12	1.00
Age, per 10 years	0.92	0.73-1.17	0.97	1.16	0.86-1.58	1.13
WHO score 1 vs 0	1.11	0.65-1.92	1.09	1.00	0.51-2.01	1.01
WHO score 2 vs 0	4.04	1.20-11.6	3.17	7.47	2.02-24.1	5.14
Serous vs non-serous	1.19	0.59-2.55	1.25	0.68	0.29-1.64	0.79
Differentiation grade 2/3/high grade NOS vs 1	5.28	1.88-22.1	2.96	5.79	1.64-37.0	2.71
FIGO stage III/IV vs I/II	3.54	1.31-10.8	2.21	2.31	0.71-9.23	1.80
Residual tumor load (yes vs no)	3.14	1.43-6.99	2.40	5.89	2.57-14.0	3.81
Treatment (NACT vs PDS)	1.56	0.68-3.47	1.52	1.45	0.60-3.41	1.47
Presence of ascites	1.63	0.96-2.84	1.50	2.76	1.43-5.63	1.96

Penalized results were based on a multivariable Cox model using a ridge penalty, where the penalization parameter lambda was derived using 10-fold cross-validation.

Abbreviations: ROMA: risk of ovarian malignancy algorithm, FIGO: International Federation of Obstetrics and Gynecology, WHO: world health organization, NOS: not other specified, NACT: neo-adjuvant chemotherapy, PDS: primary debulking surgery, DSS: disease specific survival, PFS: progression free survival, HR: hazard ratio .

Figure 1.

Kaplan-Meier curves showing **Progression Free Survival** (PFS) and Disease Specific Survival (DSS) stratified for **serum HE4** and **Risk of Ovarian Malignancy Algorithm (ROMA)** scores using a median split.

Time in years